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J.R. Clark, J.M. Patrick, Jr., J.C. Moore, & J. Forester

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16. ABSTRACT

In a preliminary test with Uca minax and U. pugilator exposed to PCBs in mud (1.04 µg PCBs/g) and a mud-sand mixture (0.37 µg PCBs/g), both species attained apparent steady state concentrations within 14 days, yielding calculated bioaccumulation factors (BAF) that ranged from 0.19 to 1.07. A definitive test with U. pugilator exposed to similar PCB concentrations in sediment produced BAF values from 0.44 to 0.71; however, the predicted time to 90% steady state ranged from 20 to 58 days. These tests demonstrate that fiddler crabs can accumulate PCBs from weathered sediments to concentrations that approximate the exposure concentrations and thus could distribute PCBs into aquatic, avian, and mammalian food webs in saltmarsh ecosystems.

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Accumulation of Sediment-Bound PCBs by Fiddler Crabs

James R. Clark, James M. Patrick, Jr., James C. Moore, and Jerrold Forester

U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, FL 32561

Polychlorinated biphenyls (PCBs) have been, and continue to be, an ecological problem because of their environmental persistence. In aquatic systems, PCBs sorb to organic matter, accumulate in sediments, and contaminate food chains. Because of the potential for causing reproductive impairment, PCBs in aquatic food chains pose a threat to human and other predators that consume fish and shellfish. Fiddler crabs accumulate PCBs from contaminated sediments (Nimmo et al. 1971) and detritus (Marinucci and Bartha 1982) and can transfer them to aquatic, avian, and terrestrial food webs when preyed upon by fishes, birds, and small mammals (Montague 1980).

The primary objective of our research was to characterize rates of PCB uptake and depuration by fiddler crabs in a simulated spoil bank habitat that contained PCBs in weathered sediment. Also, we examined whether the concentration of PCBs in substrates affected bioaccumulation by mixing PCB-laden sediments with clean sand. In a pilot study, we tested Uca pugilator, an inhabitant of relatively dry and sandy areas, and U. minax, which inhabits wetter and muddier substrates (Montague 1980), to determine if species differ in PCB uptake and depuration rates.

MATERIALS AND METHODS

Sediments contaminated with PCBs were dredged from New York Harbor, shipped at 4 C in sealed plastic buckets to the EPA laboratory in Gulf Breeze, Fl., and held at 4 C for 4 months until used in Experiment I and for 12 months until used in Experiment II. Undiluted mud and a 50/50 (v/v) mixture of mud and clean beach sand were substrates in both tests. Uncontaminated mud and mud-sand mixtures served as controls and as substitutes for contaminated mud during the depuration phase. Organic content, percentage moisture, and particle-size distributions were determined, using procedures recommended by Plumb (1981).

Pertinent factors in experimental design and test conditions are summarized in Table 1. Experiments were conducted in glass aquaria (90 cm x 25 cm x 12.5 cm) inclined 10 degrees from horizontal with an overflow standpipe opening 2 cm above the bottom at the lower end of the tank. This configuration provided

Table 1. Summary of experimental design and test conditions for exposure of fiddler crabs to PCB-contaminated sediments.

	Experiment I	Experiment II
Species tested	*Uca pugilator*, *Uca minax*	*Uca pugilator*
Test substrates	Whole mud, mud-sand, controls	Whole mud, mud-sand, controls
# experimental tanks	2 per species/substrate combination	5 per substrate type
Initial # animals/tank	26	40
# crabs/sample	2 (composite)	1 (individual)
Mean animal weight (SD)	2.56g (0.68) *U. pugilator* 2.76g (1.11) *U. minax*	1.86g (0.37) *U. pugilator*
Sample days-uptake	3, 7, 14, 21, 28, 35, 42	1, 2, 3, 5, 7, 10, 14, 28
Sample days-depuration	56, 63, 70	29, 30, 31, 33, 35, 42
Test temperature	Range 21 C to 24 C	Range 22 C to 24 C
Test salinity	Range 24‰ to 30‰ for *U. pugilator*, 5‰ for *U. minax*	Range 20‰ to 32.5‰
Photoperiod	12h Light: 12h Dark	12h Light: 12h Dark

Table 2. Physical and chemical characteristics of substrate types used in PCB bioaccumulation studies with fiddler crabs. Mean values are presented with standard deviations in parentheses.

Experiment/Substrate Type	Moisture %	Organic Matter %	PCB Concentration (µg/g dry wt)	Percentage of Total Sample Dry Weight per Size Fraction (µm)			
				>1000	1000-250	250-100	<100
I/PCB mud	58 (1.3)	6 (1.7)	1.04 (0.11)	5.5	21.2	0.9	72.4
I/PCB mud + sand	25 (0.2)	2 (0.1)	0.37 (0.07)	<0.1	65.4	1.1	33.5
I/control mud	80 (0.3)	10 (2.5)	0	4.0	52.8	1.6	41.6
I/control mud + sand	44 (0.2)	1 (1.7)	0	<0.1	70.0	2.0	18.0
II/PCB mud	47 (1.9)	5 (1.3)	0.97 (0.12)	[a]			
II/PCB mud + sand	28 (0.6)	3 (0.1)	0.55 (0.10)				
II/control mud	55 (0.3)	6 (1.0)	0				
II/control mud + sand	29 (0.2)	2 (0.2)	0				

[a] Particle size analyses not repeated for Experiment II.

a 1.5 L reservoir of water. Five liters of test substrate were added to the upper end of the tank and spread to the water's edge, providing a wicking action to moisten sediment (up to 8 cm deep) in which the crabs could burrow. A peristaltic pump delivered 0.5 L diluted filtered seawater (5 ‰) per hour to the reservoir end of U. minax tanks, while undiluted filtered seawater was siphoned at 12 L/hr into U. pugilator tanks.

Crabs were pulled or dug from their burrows for PCB analysis, rinsed in seawater, and placed in individual 250-mL glass jars that contained a raised nylon mesh floor and flowing water, but no sediment. The crabs were held overnight (14-17 hr) to permit gut evacuation and further carapace rinsing, weighed, and frozen until PCB extraction and analysis. The mesh floor prevented reingestion of crab fecal pellets, although preliminary analyses indicated no detectable PCB losses due to gut clearance or depuration during the overnight holding period. Analyses of carapaces shed by crabs overnight showed no detectable PCBs.

For both experiments, PCB concentrations in substrates were determined on Days 0 and 28 and in water on Days 7 and 28. Water samples were pumped (1 L/hr) from the test tank reservoir. One liter of water was extracted twice with 100-mL portions of nanograde petroleum ether (Burdick and Jackson Laboratories, Inc., Muskegon, MI) by shaking for 1 minute in a 2-L separatory funnel. One to 8 g of air-dried sediment were extracted with 10% acetone in petroleum ether in a Soxhlet apparatus for 4 hr. Individual or composite whole crabs were weighed and placed into a 150 mm by 25 mm screw-top test tube and homogenized three times with 10 mL of acetonitrile, using a Willems Polytron Model PT 20-ST (Brinkman Instruments, Westbury, NY). Sample extracts were concentrated and interferences removed by the methods of Schimmel et al. (1983).

PCBs were quantitated by the method of Webb and McCall (1973). Reference standard, obtained from U.S. FDA Chemical Repository, Washington, DC, was described by Sawyer (1978). Analyses were performed on a Hewlett-Packard Model 5710 gas chromatograph equipped with a ^{63}Ni electron-capture detector. Recoveries from spiked samples were greater than 90% in 32 trials; however, concentrations of PCB residues in tissues and sediments were not corrected for percentage recovery. Limits of detection were 0.1 µg/g for sediment and tissue and 0.1 µg/ℓ for water.

Statistical parameters were estimated with SAS computer programs (SAS 1982). Uptake and depuration processes were modeled using the BIOFAC model (Blau and Agin 1978).

RESULTS AND DISCUSSION

PCB content of contaminated mud was consistent between tests (Table 2). Any differences may be attributable in part to a change in moisture content prior to initiating the second experiment. No PCBs were detected in water samples in either experiment.

Apparently, the PCBs in "weathered environmental" mud were tightly bound and leached very slowly, if at all. Also, PCBs that might have leached from the mud were diluted by incoming water and flushed from the system.

The mud used in these experiments was not excessively toxic to fiddler crabs. Mortality for U. minax ranged from 2% to 14% over the contaminated substrate treatments and 6% to 10% for controls. For U. pugilator mortality ranged from 2% to 8% on contaminated substrates and 1% to 4% for controls. We attribute mortality in our test populations to cannibalism upon molting, rather than to toxic effects, because control mortality was as high as 10%, and was comparable to that observed for crabs exposed to contaminated substrates. Moreover, Rubinstein et al. (1983) determined that source mud was not acutely toxic to grass shrimp (Palaemonetes pugio), sand worms (Nereis virens), or hard clams (Mercenaria mercenaria).

Results from Experiment I (the pilot study) indicated each species reached steady state body burdens by Day 14; however, limited sample sizes and variability preclude statistical comparisons of the data. Although PCB concentration in the mud-sand mixture was only 33% that of the whole mud, both species accumulated body burdens of PCBs to similar concentrations. Mean PCB concentrations for U. pugilator at Day 28 were 0.21 µg/g from the whole mud exposure and 0.22 µg/g from the mud-sand mixture; for U. minax, the means were 0.20 µg/g and 0.30 µg/g, respectively. The crabs were placed on uncontaminated substrates on Day 42 and no PCBs were detected by Day 56 in U. pugilator or by Day 63 in U. minax.

Experiment II utilized U. pugilator only and incorporated more replicates per sample period to provide statistical confidence in describing PCB bioaccumulation from contaminated sediments. Sample data and uptake and depuration curves drawn from the BIOFAC model are presented in Figure 1. The crabs exposed to either sediment type accumulated comparable concentrations of PCBs by Day 21, although there was a high degree of variability in the data. PCB concentrations in crabs exposed to the whole mud appeared to reach steady state later than those from the mud-sand mixture. After 14 days, PCB depuration was nearly complete for crabs exposed to the mud-sand mixture while 3 of the 5 crabs sampled from the whole mud exposure had quantifiable PCB concentrations.

A PCB bioaccumulation factor (BAF) was calculated for each species-substrate combination, using the mean body-burden for Day 28 divided by the substrate PCB concentration. Day 28 was chosen for comparability between both tests. An additional estimate of PCB bioavailability was computed from the BIOFAC model predictions of body burdens at Day 28. Steady state BAFs, calculated as the ratio of the uptake rate and the depuration rate, also were derived from BIOFAC. These data are presented in Table 3 with a BIOFAC estimate of time necessary to reach the

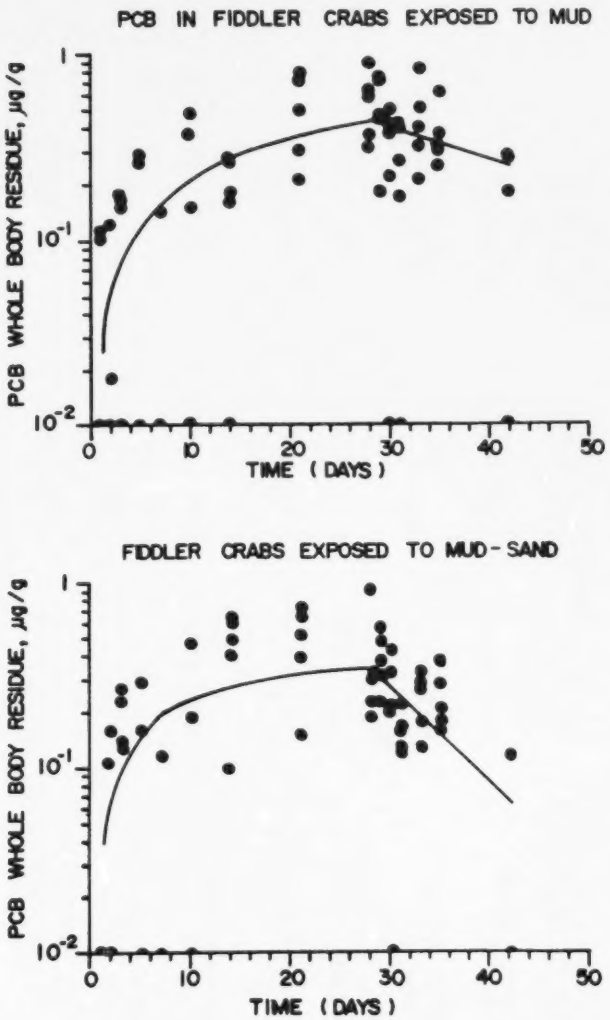

Figure 1. Uptake and depuration of PCBs by fiddler crabs, Uca pugilator, exposed to PCB-contaminated mud (0.97 µg/g dry weight) and a mud-sand mixture (0.55 µg/g dry weight). Five crabs were sampled per time period, animals were transferred to clean sediment on day 28 for depuration. Concentrations are µg/g wet weight for whole crabs.

Table 3. Means for measured and calculated bioaccumulation factors (BAF) for fiddler crabs exposed to PCB-contaminated substrates. Standard deviations are in parentheses.

Test Substrate	Data Used in Numerator of BAF Calculation	Experiment I		Experiment II
		U. minax	U. pugilator	U. pugilator
Mud	Measured burden at day 28	0.19 (0.095)	0.20 (0.136)	0.58 (0.245)
	BIOFAC prediction at day 28[a]	0.42	0.21	0.44
	BIOFAC prediction at steady state[b]	0.42 (0.118)	0.21 (0.042)	0.66 (0.263)
	Time to 90% steady state	12 days (2.0)	5 days (0.3)	58 days (20.7)
Mud-Sand	Measured burden at day 28	0.79 (0.248)	0.59 (0.355)	0.71 (0.536)
	BIOFAC prediction at day 28[a]	1.07	0.71	0.62
	BIOFAC prediction at steady state[b]	1.07 (0.265)	0.71 (0.116)	0.64 (0.122)
	Time to 90% steady state	12 days (2.0)	5 days (0.3)	20 days (2.5)

[a] Only a single residue concentration predicted for Day 28
[b] Standard deviation derived by BIOFAC model as estimate of goodness of fit (Blau and Agin 1978)

predicted 90% steady state concentrations.

Discrepancies between BAFs calculated from measured residues or BIOFAC model predictions result from the model's use of the entire uptake and depuration data base, thereby reducing the bias associated with selecting data from a particular day as representative of steady state concentrations. BAFs calculated from measured or predicted body burdens generally agree and most have considerable variances. Because of the variability of PCB concentrations within crab populations, little significance can be attributed to higher BAF values calculated for U. minax compared to U. pugilator, especially in light of our test results indicating that BAFs of U. pugilator in Experiment II were similar to those of U. minax in Experiment I. Also, differences between calculated BAFs for the mud and mud-sand mixture in Experiment I were not observed in Experiment II.

The BAFs in Table 3 range between 0.19 and 1.07. Nimmo et al. (1971) exposed U. pugilator to PCB-contaminated sediments for 30 days. From those data, we calculated BAFs that ranged from 0.6 to 1.3. Similar values have been reported for Mercenaria mercenaria (<0.5 BAF), Palaemonetes pugio (<0.5 BAF), and Nereis virens (0.15 to 1.59 BAF) exposed to PCB-contaminated sediments (Rubinstein et al. 1983). These BAFs fall considerably below the bioconcentration factors of 10^4 to 10^5 commonly reported for waterborne exposures for fishes and invertebrates (Thomann 1981) and demonstrate that sediment-bound PCBs are accumulable by foraging fiddler crabs, but not to the extent one would expect from exposure to waterborne PCBs.

Although use of fiddler crabs in bioavailability testing has considerable ecological application, much of the variability in our data can be attributed to their complex behavior. The densitites of our test populations did not exceed those reported from field observations (Montague 1980); however, by confining the crabs to a closed system, we may have affected individual behavior and mobility and thus the variability of the results. Our data have established that fiddler crabs have the potential to accumulate PCBs rapidly from contaminated sediments and to develop body burdens similar to concentrations in sediment. Exposure to sediment-bound PCBs did not yield body burdens in fiddler crabs as high as might be expected, based on PCB bioconcentration from waterborne exposures. However, contaminated sediments must be taken into account when evaluating environmental exposures to PCBs. Because fish, birds, or mammals feed upon fiddler crabs, sediment-bound PCBs may be accumulated to higher concentrations in the upper trophic levels of wetland food webs.

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